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REVIEW

Emerging immune-based technologies for high-grade gliomas

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ABSTRACT

Introduction: The selection of a tailored and successful strategy for high-grade gliomas (HGGs) treatment is still a concern. The abundance of aberrant mutations within the heterogenic genetic landscape of glioblastoma strongly influences cell expansion, proliferation, and therapeutic resistance. Identification of immune evasion pathways opens the way to novel immune-based strategies. This review intends to explore the emerging immunotherapies for HGGs. The immunosuppressive mechanisms related to the tumor microenvironment and future perspectives to overcome glioma immunity barriers are also debated.

Areas covered: An extensive literature review was performed on the PubMed/Medline and ClinicalTrials.gov databases. Only highly relevant articles in English and published in the last 20 years were selected. Data about immunotherapies coming from preclinical and clinical trials were summarized.

Expert opinion: The overall level of evidence about the efficacy and safety of immunotherapies for HGGs is noteworthy. Monoclonal antibodies have been approved as second-line treatment, while peptide vaccines, viral gene strategies, and adoptive technologies proved to boost a vivid antitumor immunization. Malignant brain tumor-treating fields are ever-changing in the upcoming years. Constant refinements and development of new routes of drug administration will permit to design of novel immune-based treatment algorithms thus improving the overall survival.

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CAR T; glioblastoma; high-grade glioma; immunotherapy; monoclonal antibodies; oncolytic virus; peptide vaccine; tumor microenvironment

1. Introduction

High-grade gliomas (HGGs) are prevalent brain tumors and glioblastoma (GBM) is the most fast-growing and deadly accounting for 60% of primary gliomas in the adult population [1–7]. Since 2005, the Stupp standard therapy for HGGs consisted of the maximum surgical resection followed by concurrent adjuvant chemo and radiotherapy [8,9]. Despite the assessed efficacy of the conventional protocol, no improvement in the tumor remission was achieved. The median overall survival remains at 14–16 months and the 5-year survival rate persists under 10% after diagnosis [10].

The grim prognosis and high rate of recurrence are consequential to the intrinsic tumor heterogeneity, intense self-renewal activity, abnormal vascular growth, and immune evasion mechanisms [11–19].

Recent evidence from literature identifies the genesis of glioma resilience in the immune suppressive mechanisms. Glioma cells secrete chemokines, cytokines, and growth factors which promote the recruiting of lymphocytes, T cells CD4+, CD8+, Treg, NK cells, monocytes, and macrophages [20,21]. The infiltration of a wide range of immune cells builds up an immune niche, namely the glioma microenvironment. The crosstalk between immune cells and matrix cells manages the cancer growth, spreading, and mechanisms of tumor resistance [22]. Hence, the glioma microenvironment is strictly related to patients' prognosis, survival, and response to therapy.

On these assumptions, the new therapeutic frontiers focused on the design of strategies aimed at modifying tumor genomics, manipulating the glioma-related immune microenvironment, thus boosting the antitumor immunity.

Advances in translational medicine and the engineering of molecular vehicles, such as nanoparticles or attenuated viruses, allowed to concretize of novel immunotherapies. The most promising approaches included the active and adoptive immune technologies, both targeted to the activation of the host's immune system.

The present article overviews the emerging immune-based technologies for the HGGs treatment focusing on the alkylating agents, monoclonal antibodies, vaccines, gene techniques, immunomodulatory approaches, and adoptive immunotherapies. The composition of the immunosuppressive glioma microenvironment, the role of each immune infiltrate in the mechanisms of therapeutic resistance, the limitations, and the future challenges are furtherly discussed.

2. Classification of immunotherapies for high-grade gliomas

As previously described by our group, the immune-based technologies were categorized according to the molecular targets, mechanism of action, and drugs [23–32]. Two treatment arms were outlined, namely active and adoptive approaches. Active immunotherapies were designed to directly lyse cancer cells and/or trigger the antitumor immune

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Article highlights

- The high-grade gliomas aggressiveness and resistance to conventional therapies are owed to the genomic heterogeneity and adaption mechanisms recognized in the tumor immunosuppressive microenvironment.
- Immunotherapies strive to manipulate and manage pathways of glioma immune escape.
- Bevacizumab, an anti-VEGF-A monoclonal antibody, is approved as a second-line treatment for recurrent glioblastoma.
- The vaccinations proved to be useful in enhancing the antitumor immune response, although not leading to an increase in survival.
- Gene therapies, based on viral vectors, or immunomodulatory gene technologies showed excellent results in vitro studies, but also in some clinical trials.
- Immunogenomics and identification of specific tumor antigens let the design of adoptive immunotherapies. The CAR T cells are engineered to selectively target glioma cells resulting in oncolysis.
- A better understanding of the resilience pathways will be vital in improving immune-based therapies for brain tumors. The future challenge is the planning of combined protocols to improve the overall and progression-free survival of HGGs patients.

cascade. Active treatments include checkpoint inhibitors, such as the alkylating drugs and monoclonal antibodies; vaccines; and some gene-based technologies comprehending oncolytic virotherapies and immunomodulatory strategies.

The adoptive immunotherapies provide the transfer of autologous or allogeneic engineered T, natural killer (NK), and natural killer T (NKT) cells with the aim to restore the host antitumoral immune response. Table 1 summarizes the classification of immune-based therapies for HGGs (Table 1).

2.1. Active immunotherapies

2.1.1. Checkpoint inhibitors

Checkpoint inhibitors (CPIs) are at the vanguard of immunotherapies showing real life-saving benefits for GBM patients. CPIs are classified as chemotherapy drugs, which act during distinct phases of the cell cycle, and monoclonal antibodies (MAbs).

Alkylating drugs are chemotherapy compounds that methylate DNA azotate bases avoiding the binding of double-stranded DNAs and glioma cell proliferation. Temozolomide (TMZ), combined with surgery and radiotherapy, remains the first-line systemic drug in the treatment of HGGs [8,9]. It is administered orally as a prodrug, reaches appropriate concentrations, and passes the blood–brain barrier (BBB) [33–36]. TMZ achieves the tumor site where it is spontaneously converted into its active form, the 5-3-methyltriazen-1-yl-imidazole-4-carboxamide (MTIC). MTIC methylates guanine or adenine DNA bases on the N7-/O6- or N3-site, respectively, resulting in base pair mismatch, DNA rupture, and glioma cell apoptosis [37–39]. Accordingly, the therapeutic efficacy of TMZ is strictly reliant on the mechanisms of DNA repair [40,41]. The O6-methylguanine-DNA methyltransferase enzyme, known as MGMT, demethylates the damaged O6-methylguanine nucleotide, thus repairing chromatin and

Table 1. Classification of Immunotherapies for High-Grade Gliomas.

Immunotherapies				
Active	Checkpoint inhibitors	Alkylating agent		TMZ
		MAbs		BVZ
				Ipilimumab
				Nivolumab
				Pembrolizumab
				Relatlimab
				Rindopepimut
	Vaccine			R123H/IDH1
				IMA950
				HSPCC-96
				DCVax-Brain
				PerCellVac2
	Gene-based therapy	*Oncolytic viruses*		*oHSV*
				CRAd
				MV
				PVS-RIPO
				oNDV
				Reolysin
		Immunomodulatory Genes		IL-4
				IL-12
				IFNβ/γ
Adoptive	T cell	CAR T	TCR T	
				EGFRvIII
				IL-13Ra
				2HER2
				EphA2
	NK cell		Allogenic NK	
			DNRII	
			CAR NK cells	
			ADCC	
			Anti-KIR Abs	
	Hybrid	CD1d-restricted NKT cellsALECSAT		

ADCC: antibody-dependent cellular cytotoxicity; ALECSAT: autologous lymphoid effector cells specific against tumor; Anti-KIR Abs: antibody-mediated blocking of KIR; BVZ: bevacizumab; CAR T: chimeric antigen receptor; DC: dendritic cell; DNRII: dominant-negative receptor II; EGFRIII: epidermal growth factor receptor variant III; EphA2: erythropoietin-producing hepatocellular carcinoma A2; HER2: human epidermal growth factor 2; HSPCC: heat-shock proteins peptide complex; IL-13Ra2: interleukin-13 receptor α2; MAbs: monoclonal antibodies; NK: natural killer cells; NKT: T lymphocyte-natural killer cells; oNDV: oncolytic Newcastle disease virus; T: T lymphocyte; TMZ: temozolomide.

keeping the cell cycle going [42,43]. The methylation status of the CpG Islands, within the MGMT promoter, and the consequent enzyme expression is a relevant prognostic factor for chemo response to alkylating agents [44–48]. Figure 1 reports the TMZ pharmacodynamics and MGMT mechanism of chemoresistance (Figure 1).

Furthermore, the TMZ exerts a specific immunomodulatory effect based on its dosage. In 2011, Mitchell and colleagues conducted a preclinical study with the employment of increasing doses of TMZ in murine models, combined with immunotherapy. They demonstrated the antitumor effect of TMZ and its influence on the host immune response by causing lymphopenia. The transient TMZ-induced lymphopenia triggered the immune cascade raising the regulatory T cells (Tregs) activity [49]. Also, the systemic administration of 10 lower concentrations of TMZ in GL261 glioma-bearing mice was demonstrated to boost the NK cells within the tumor microenvironment. The rationale lies in the overexpression of the multidrug resistance protein Abcc3 (ATP Binding Cassette Subfamily C Member 3) on NK cells' surfaces, liable of chemoresistance to TMZ [50].

The DENDR1 clinical trial tested the TMZ in combination with dendritic cell immunotherapy for GBM patients (#NCT04801147). Results reported a decrease of peripheral CD8 + T cells simultaneously with an overactivation of the Abcc3- expressing NK cells [50]. A further preclinical study tried the intra-tumoral administration of TMZ through a micro-osmotic pump in GL261 models. A local increase of immune effector cells was demonstrated [51].

All above-mentioned data demonstrated both systemic and sited administration of TMZ results in increased immunostimulant mechanisms and strengthening of concomitant immunotherapies [49–52].

The MAbs are tailored proteins acting as human immunoglobulins. MAbs target specific molecular ligands and mimic the host antitumoral response.

The backbone of this strategy is the bevacizumab (BVZ). It binds the vascular endothelial growth factor A (VEGF-A) and blocks its activation through the VEGF tyrosine kinase receptors, upregulated on the surface of glioma cells [53–60]. Impound of VEGF-A inhibits cell growth, aberrant tumor vasculogenesis, and reduces the vascular density and permeability [54,61–65].

BVZ also plays a pivotal role in the regulation of the antitumoral immune response. VEGF-A supports an immunosuppressive glioma microenvironment by impeding dendritic cells' maturation and enhancing the proliferation of Tregs [63,66]. BVZ-mediated blockade of the VEGF proved to establish a favorable immune niche, boost the host immune response through a synergistic effect with cell-lytic chemotherapy, and inhibit glioma stem cells [64,67,68].

In 2009, BVZ was approved by the Food and Drug Administration (FDA) as a second-line treatment for recurrent GBMs [69,70] (Figure 2(a)).

The programmed cell death protein 1 (PD-1), expressed on active lymphocytes, bounds the programmed cell death protein ligands (PD-L1/2) exposed on the surface of glioma cells. This linkage prevents the CD4+ and CD8+ activity and sustains the mechanisms of immune escape [71,72]. The nivolumab, a MAb IgG directed against PD-1, was tried in many clinical trials on recurrent HGGs, but the results are still preliminary [73–75] (Figure 2(b)). In 2019, Schalper and his group conducted a single-arm phase II clinical trial testing the nivolumab as a neo- and adjuvant treatment for GBMs (#NCT02550249). They administered the drug before and after surgery in 30 cases, compared to a control group treated with the standard protocol. Although not showing effective survival benefits,

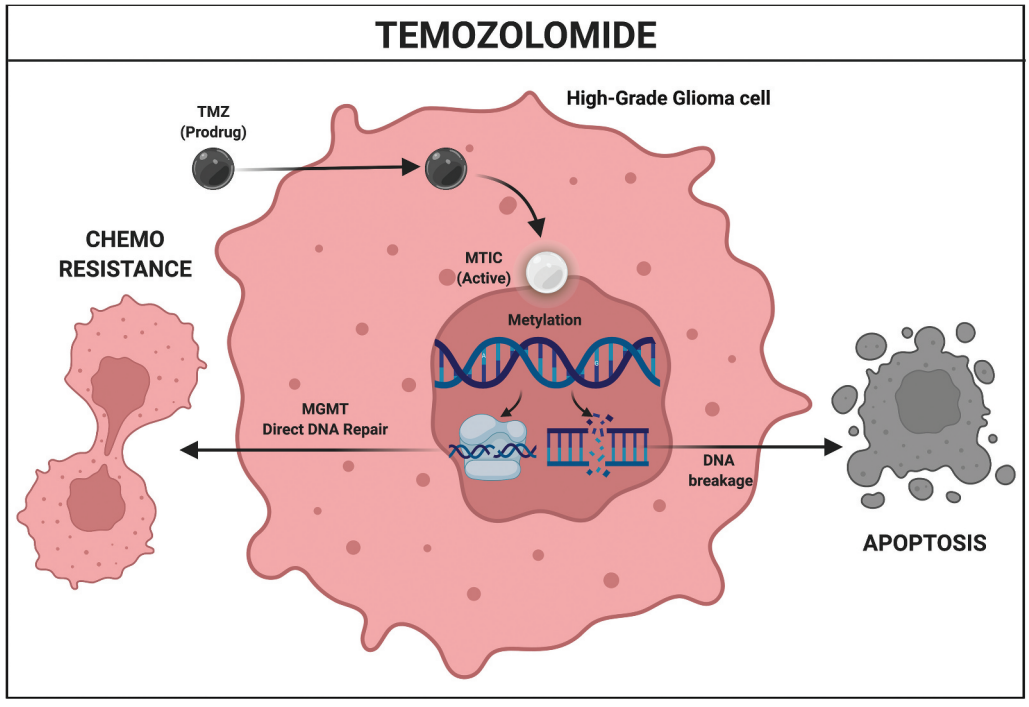

Figure 1. The image depicts the pharmacodynamics of temozolomide (TMZ). It is orally administered as a prodrug, converted at the tumor site into the 5-3-methyltriazen-1-yl-imidazole-4-carboxamide (MTIC), the active form. MTIC methylates bases leading to DNA breakage and apoptosis. If the O6-methylguanine-DNA methyltransferase enzyme (MGMT) demethylates the nucleotides and repairs chromatin, the glioma cell develops chemoresistance to TMZ.

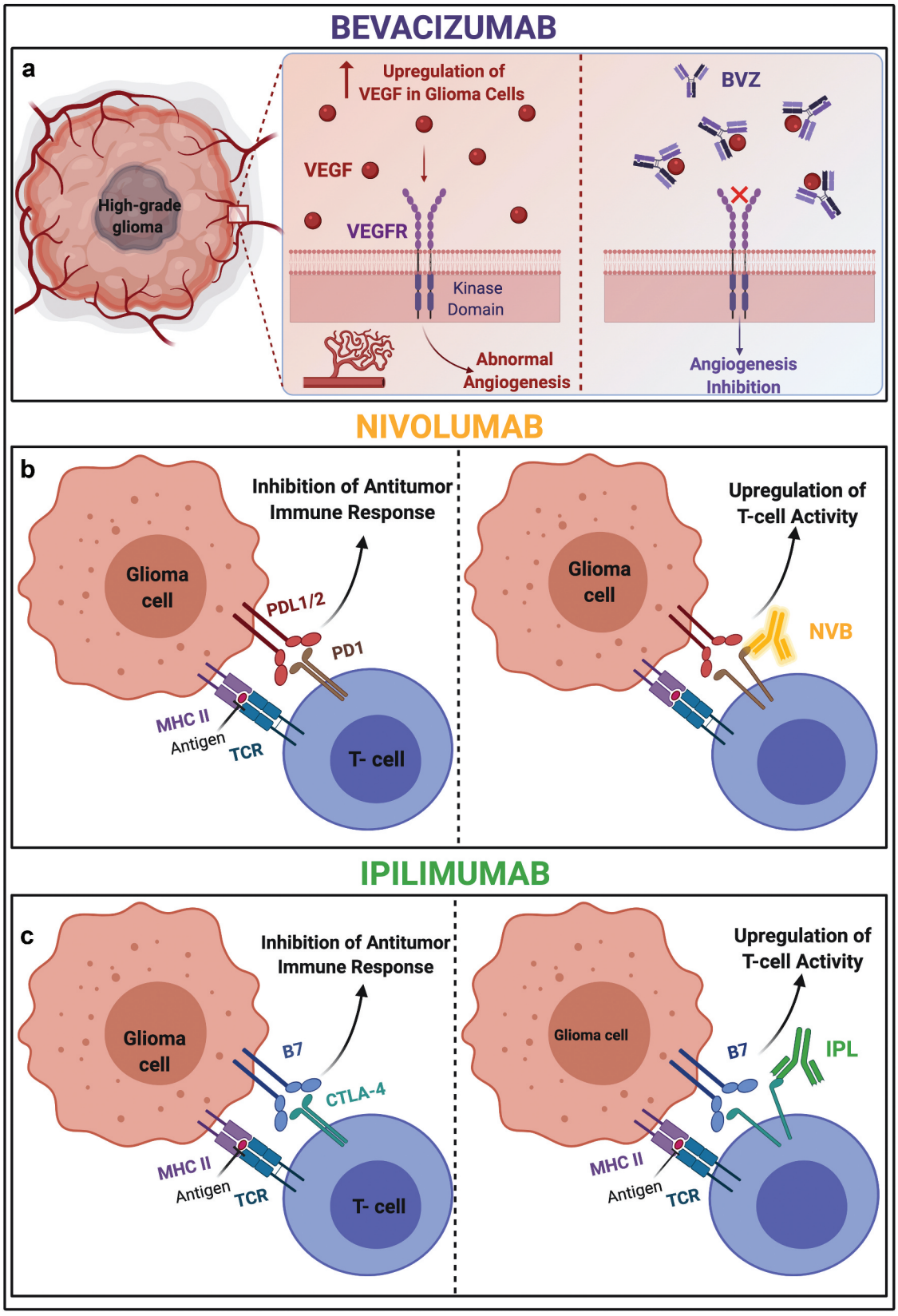

Figure 2. Mechanisms of action of MAbs, (a) Bevacizumab (BVZ), (b) Nivolumab (NVB), and (c) Ipilimumab (IPL) were represented.

(A) BVZ blocks the vascular endothelial growth factor A (VEGF-A) preventing its binding with the vascular endothelial growth factor tyrosine kinases receptor (VEGFR), overexpressed on glioma cells. BVZ-mediated blockade of the VEGF inhibits the abnormal angiogenesis within the tumor microenvironment; (B) NVB directly binds the programmed cell death protein 1 (PD-1) on the active T-cells, thwarting the CD4+ and CD8+ activity and supporting the immune escape; (C) IPL binds the cytotoxic T-lymphocyte antigen 4 (CTLA-4), a T-cell receptor, deputy to the shutdown of the immune cascade. This linkage permits the upregulation of T-cells activity.MHC: Major Histocompatibility Complex; PDL-1/2: Programmed Cell Death Protein-Ligand 1/2; TCR: Transgenic T Cell Receptor.

administration of nivolumab was revealed to enhance the local chemokine infiltration and promote the immune tumor microenvironment [75].

Further clinical trials tested the nivolumab and BVZ as a combined protocol for HGGs treatment (#NCT03890952, #NCT03743662, #NCT03452579). Results were not noteworthy. In 2020, Reardon et al. completed a phase 3 randomized clinical trial comparing the effects of nivolumab to BVZ in patients with recurrent GBMs (#NCT02017717). After treatment with TMZ and radiotherapy, 369 patients with relapsed GBM were enrolled and divided into two groups treated with nivolumab (3 mg/kg) or BVZ (10 mg/kg) for 2 weeks. Results showed equivalent average survival between the two groups, with an excellent safety profile for nivolumab [74].

In 2021, pembrolizumab (Mab targeting PD-L1) was employed in phase II clinical trials, as monotherapy or combined with BVZ, for recurrent GBMs. No significant increase in survival was reported [76]. In 2021, Reardon and his group conducted the phase I 'KEYNOTE-028' clinical study to prove the antitumor effectiveness and tolerance profile of pembrolizumab in PD-L1-positive GBMs (#NCT02054806). Pembrolizumab (10 mg/kg) was administered every 2 weeks for 2 years. Results reported a durable efficacy as monotherapy, with a low toxicity rate [77]. Two recruiting clinical trials are employing the pembrolizumab for recurrent pediatric tumors, including HGGs (#NCT02359565; #NCT03173950). Results are still pending.

Atezolizumab, a PD-L1 inhibitor, demonstrated potential therapeutic effects in a phase II trial for recurrent HGGs, especially with a high level of CD4+ within the tumor microenvironment [78].

Several preclinical studies investigated the role of T-cell immunoglobulin mucin-3 (TIM-3) expression as responsible for resistance to TMZ and negative regulator of lymphocyte activity [79,80].

A promising strategy may consist of TIM-3 repression via engineered siRNA. The downregulation of TIM-3 noticeably reduced glioma cell growth and increased the therapeutic effect of TMZ [81,82]. The effects of TIM-3 blockades also showed a synergic efficacy in combination with anti-PD-1, resulting in durable antitumor immunity [83]. More clinical studies are needed to develop antibodies against TIM-3, expressed on CD4+ and CD8 + T cells.

A further innovative checkpoint inhibitory molecule is the T cell immunoreceptor with Ig and ITIM domains (TIGIT). TIGIT is potentially involved in cancer immunity, being overexpressed on tumor-infiltrating T cells, Tregs, and NK cells [84].

Anti-PD-1 and anti-TIGIT combination therapy demonstrated an anticancer effect in glioma murine models both in improving survival and repressing suppressive Tregs and tumor-infiltrating cell activity [85].

The cytotoxic T-lymphocyte antigen 4 (CTLA-4), a T lymphocyte receptor, physiologically downregulates the immune system. The MAb ipilimumab was designed to block the CTLA-4, thus preventing the shutdown of the immune cascade (Figure 2(c)). In 2016, the ipilimumab was administered with BVZ or TMZ in HGGs treatment. Data showed a good tolerance profile without concrete efficacy in patients' survival [86,87]. In 2018, Omuro et al. tested in phase III clinical trial 'CheckMate 143' the ipilimumab combined with nivolumab for 40 GBM patients. They reported a better safety profile for nivolumab as monotherapy, compared with nivolumab and ipilimumab, but no survival benefits were noted [88]. An ongoing trial is trying nivolumab ± ipilimumab as neo- and adjuvant therapy, but the results are still pending (#NCT04606316).

The lymphocyte activation gene-3 (LAG3), found on the surface of lymphocytes, regulates CD4+ activity and homeostasis. LAG3 was selected as a molecular target for its overexpression within the glioma microenvironment [89–91]. The relatlimab, MAbs anti-LAG3, combined with nivolumab, is currently under experimentation in phase I clinical trial. The estimated completion date is April 2022 (#NCT02658981).

Furthermore, the APX005M, a humanized IgG vs the CD40, has been employed in phase I clinical trial for recurrent or refractory pediatric brain tumors, showing good preliminary results. The estimated study completion date is September 2022 (#NCT03389802).

Metabolic enzymes involved in tryptophan (Trp) catabolism, arginase, and CD73, modulate the immune cell function and may be considered as immune checkpoint molecules.

Pathways of Trp are crucial in supporting tumorigenesis [92]. The essential amino acid Trp is catabolized by the rate-limiting enzyme like the indoleamine 2,3-dioxygenase (IDO), expressed on 90% of HGGs. IDO activity results in Trp depletion and accumulation of immunosuppressive downstream catabolites within the tumor microenvironment [93]. Competitive inhibitors of IDO were designed aiming to improve the efficacy of standard protocol. The 1-methyl L-tryptophan (1MT), an IDO inhibitor, was employed – after interferon-γ stimulation – combined with TMZ, bischloroethylnitrosourea, etoposide, and cisplatin. Excellent anticancer activity and improvement in chemotherapy efficacy were reported in mouse models [94–97].

In 2020, phase I/II clinical trials explored the effect of 1-MT combined with chemotherapy regimens in both pediatric and adult HGGs gliomas. Results proved a good safety profile and relevant efficacy (#NCT02052648, #NCT02502708).

Other studies focused on a dioxygenase involved in Trp catabolism, namely the tryptophan 2,3 dioxygenase (TDO) [92,98]. An oral TDO inhibitor, 3-(2-(pyridyl)ethenyl)indole (680C91), demonstrated immune boosting activity and an enhancement of chemotherapy [99–101].

Furthermore, the CD73, an extracellular nucleotidase implicated in adenosine metabolism, is involved in resistance to standard therapy and gliogenesis via cell proliferation and rearrangement of the extracellular matrix. The CD73 downregulation, via delivery of siRNA-CD73, proved to reduce tumor size by 45% and inhibit glioma progression in vivo models [102–105].

Nasal administration of cationic nanoemulsion mixed with CD73-siRNA demonstrated to suppress the glioma cell proliferation and alter the tumor immune response [106,107].

Table 2 summarizes the main clinical trials on checkpoint inhibitors for HGGs treatment (Table 2).

Even considering the undeniable advances in translational medicine which allowed the engineering of novel chemotherapeutic agents and mAbs, some limitations persist. Failure of

Table 2. Main clinical trials on checkpoint inhibitors for HGGs.

#	ClinicalTrials.gov Identifier	Title	Status	Phase	Diseases	# of Pts. Enrolled	Treatment	Locations
1	NCT03011671	Study of Acetazolamide with Temozolomide in Adults with Newly Diagnosed or Recurrent Malignant Glioma	Suspended	I	Malignant Glioma of Brain	24	Acetazolamide, Temozolomide	USA
2	NCT02416999	Ultra-low Dose Bevacizumab Plus Temozolomide for Recurrent High-grade Gliomas	Unknown	NA	Recurrent High-grade Glioma	30	Ultra-low dose Bevacizumab, Temozolomide	CHN
3	NCT01891747	A Phase I Study of High-dose L-methylfolate in Combination with Temozolomide and Bevacizumab in Recurrent High-Grade Glioma	Active, not recruiting	I	Malignant Glioma	12	Bevacizumab, Temozolomide, Vitamin C	USA
4	NCT04267146	Nivolumab in Combination with Temozolomide and Radiotherapy in Children and Adolescents with Newly Diagnosed High-grade Glioma	Recruiting	I, II	High Grade Glioma	40	Nivolumab, Temozolomide, Radiotherapy	FR
5	NCT00782756	Bevacizumab, Temozolomide and Hypofractionated Radiotherapy for Patients with Newly Diagnosed Malignant Glioma	Completed	II	Brain Cancer, Malignant Glioma	40	Radiotherapy, Temozolomide, Bevacizumab	USA
6	NCT04547621	HSRT and IMRT Chemoradiotherapy for Newly Diagnosed GBM	Active, not recruiting	I, II	Glioma, Malignant	50	Radiation, Temozolomide	CHN
7	NCT01390948	A Study of Bevacizumab (Avastin) in Combination with Temozolomide and Radiotherapy in Pediatric and Adolescent Participants With High-Grade Glioma	Completed	II	High Grade Glioma	124	Bevacizumab, Radiotherapy, Temozolomide	A
8	NCT00660621	A Phase II Study of Gliadel, Concomitant Temozolomide and Radiation, Followed by Dose Dense Therapy with Temozolomide Plus Bevacizumab for Newly Diagnosed Malignant High-Grade Glioma	Unknown	II	Glioma	40	Temozolomide, Bevacizumab	USA
9	NCT03633552	Efficacy of Two Temozolomide Regimens in Adjuvant Treatment of Patients with Brain High Grade Glioma	Recruiting	III	Glioblastoma Multiforme Anaplastic Astrocytoma	62	Temozolomide	IR
10	NCT01105702	Temodar (Temozolomide), Bevacizumab, Lithium and Radiation for High Grade Glioma	Terminated	II	Brain Cancer	28	Temozolomide, Bevacizumab, Lithium Carbonate, Radiation	USA
11	NCT01740258	Bevacizumab Beyond Progression (BBP)	Completed	II	Malignant Glioma, Glioblastoma, Gliosarcoma	68	Radiation Therapy, Temozolomide, Bevacizumab	USA
12	NCT01478321	Efficacy of Hypofractionated XRT w/Bev. + Temozolomide for Recurrent Gliomas	Terminated	II	Adult Anaplastic Astrocytoma Ependymoma Oligodendroglioma Glioblastoma	54	Temozolomide, Bevacizumab Hypofractionated radiation therapy	USA
13	NCT00943826	A Study of Bevacizumab (Avastin®) in Combination with Temozolomide and Radiotherapy in Participants with Newly Diagnosed Glioblastoma	Completed	III	Glioblastoma	921	Bevacizumab, Temozolomide Radiation therapy	USA
14	NCT00884741	Temozolomide and Radiation Therapy with or Without Bevacizumab in Treating Patients with Newly Diagnosed Glioblastoma	Completed	III	Glioblastoma, Gliosarcoma, Supratentorial Glioblastoma	637	Radiation Therapy, Temozolomide, Bevacizumab	USA
15	NCT01046279	Hypertension Monitoring in Glioma Patients Treated with Bevacizumab	Terminated	NA	Glioma	40	Bevacizumab	ZH
16	NCT00271609	Bevacizumab for Recurrent Malignant Glioma	Completed	II	Recurrent High-Grade Gliomas	88	Bevacizumab	USA
17	NCT02833701	Bevacizumab and Ascorbic Acid in Patients Treating with Recurrent High-Grade Glioma	Terminated	I	Glioblastoma, Glioma	9	Ascorbic Acid, Bevacizumab	USA
18	NCT00595322	Bevacizumab in the Radiation Treatment of Recurrent Malignant Glioma	Completed	NA	Recurrent Malignant Gliomas, Primary Brain Tumor	25	Bevacizumab, Radiation	USA
19	NCT00337207	Bevacizumab in Treating Patients with Recurrent or Progressive Glioma	Completed	II	Central Nervous System Tumors	55	Bevacizumab	USA
20	NCT01091792	Exploratory Study of the Modulation of the Immune System by VEGF Blockade in Patients with Glioblastoma Multiforme (GBM)	Completed	I	Glioblastoma Multiforme	13	Bevacizumab	USA

(*Continued*)

Table 2. (Continued).

#	ClinicalTrials.gov Identifier	Title	Status	Phase	Diseases	# of Pts. Enrolled	Treatment	Locations
21	NCT00883298	Bi-weekly Temozolomide Plus Bevacizumab for Adult Patients with Recurrent Glioblastoma Multiforme	Completed	II	Recurrent Glioblastoma Multiforme Recurrent Gliosarcoma	30	Temozolomide, Bevacizumab	USA
22	NCT01811498	Repeated Super-Selective Intraarterial Cerebral Infusion of Bevacizumab (Avastin) for Treatment of Newly Diagnosed GBM	Active, not recruiting	I, II	Glioblastoma Multiforme, Brain Tumor	25	Bevacizumab	USA
23	NCT01730950	Bevacizumab With or Without Radiation Therapy in Treating Patients with Recurrent Glioblastoma	Active, not recruiting	II	Adult Giant Cell Glioblastoma, Glioblastoma, Adult Gliosarcoma Recurrent Adult Brain Tumor	182	Bevacizumab, Radiation therapy	USA
24	NCT02761070	Bevacizumab Alone Versus Dose-dense Temozolomide Followed by Bevacizumab for Recurrent Glioblastoma, Phase III	Recruiting	III	Recurrent Glioblastoma	146	Temozolomide, Bevacizumab	J
25	NCT01209442	Hypofractionated Intensity-Modulated Radiation Therapy With Temozolomide and Bevacizumab for Glioblastoma Multiforme	Completed	II	Glioblastoma Multiforme	30	Bevacizumab, Temozolomide Radiation Therapy	USA
26	NCT01125046	Bevacizumab in Treating Patients with Recurrent or Progressive Meningiomas	Completed	II	Central Nervous System Tumors	50	Bevacizumab	USA
27	NCT01526837	Bevacizumab (Avastin) Into the Tumor Resection Cavity in Subjects With Glioblastoma Multiforme at First Recurrence	Terminated	I	Glioblastoma Multiforme	1	Bevacizumab	USA
28	NCT01443676	Avastin Plus Radiotherapy in Elderly Patients with Glioblastoma	Completed	II	Glioblastoma	75	Bevacizumab, Radiation therapy	USA
29	NCT00590681	Bevacizumab and Temozolomide Following Radiation and Chemotherapy for Newly Diagnosed Glioblastoma Multiforme	Completed	II	Glioblastoma Multiforme	62	Bevacizumab, Temozolomide	USA
30	NCT03925246	Efficacy of Nivolumab for Recurrent IDH Mutated High-Grade Gliomas	Active, not recruiting	II	High Grade Glioma, Brain Cancer	43	Nivolumab	FR
31	NCT00345163	A Study to Evaluate Bevacizumab Alone or in Combination with Irinotecan for Treatment of Glioblastoma Multiforme (BRAIN)	Completed	II	Glioblastoma	167	Bevacizumab, Irinotecan	NA
32	NCT03890952	Translational Study of Nivolumab in Combination with Bevacizumab for Recurrent Glioblastoma	Recruiting	II	Recurrent Adult Brain Tumor	40	Nivolumab, Bevacizumab	DNK
33	NCT01498328	A Study of Rindopepimut/GM-CSF in Patients with Relapsed EGFRvIII-Positive Glioblastoma	Completed	II	Glioblastoma	127	Rindopepimut (CDX-110) with GM-CSF Bevacizumab, KLH	USA
34	NCT03743662	Nivolumab With Radiation Therapy and Bevacizumab for Recurrent MGMT Methylated Glioblastoma	Recruiting	II	Glioblastoma	94	Re-irradiation, Bevacizumab Nivolumab, Re-resection	USA
35	NCT03452579	Nivolumab Plus Standard Dose Bevacizumab Versus Nivolumab Plus Low Dose Bevacizumab in GBM	Active, not recruiting	II	Glioblastoma	90	Nivolumab, Standard/Reduced Dose Bevacizumab	USA
36	NCT02550249	Neoadjuvant Nivolumab in Glioblastoma	Completed	II	Glioblastoma Multiforme	29	Nivolumab	ES
37	NCT04195139	Nivolumab and Temozolomide Versus Temozolomide Alone in Newly Diagnosed Elderly Patients With GBM	Recruiting	II	Glioblastoma Multiforme	102	Nivolumab, Temozolomide	A
38	NCT02667587	An Investigational Immuno-Therapy Study of Temozolomide Plus Radiation Therapy with Nivolumab or Placebo, for Newly Diagnosed Patients with Glioblastoma (GBM, a Malignant Brain Cancer)	Active, not recruiting	III	Brain Neoplasms	693	Nivolumab, Temozolomide, Radiotherapy	USA
39	NCT02617589	An Investigational Immuno-Therapy Study of Nivolumab Compared to Temozolomide, Each Given with Radiation Therapy, for Newly diagnosed Patients with Glioblastoma	Active, not recruiting	III	Brain Cancer	560	Nivolumab, Temozolomide, Radiotherapy	USA
40	NCT01213407	Dendritic Cell Cancer Vaccine for High-grade Glioma	Completed	II	Glioblastoma Multiforme	87	Trivax, Temozolomide, Surgery, Radiotherapy	AU
41	NCT02529072	Nivolumab With DC Vaccines for Recurrent Brain Tumors	Completed	I	Malignant Glioma, Astrocytoma	6	Nivolumab	USA
42	NCT03718767	Nivolumab in Patients With IDH-Mutant Gliomas with and Without Hypermutator Phenotype	Recruiting	II	Malignant Glioma of Brain	95	Nivolumab	USA
43	NCT01480479	Phase III Study of Rindopepimut/GM-CSF in Patients with Newly Diagnosed Glioblastoma	Completed	III	Malignant Glioma of Brain	745	Rindopepimut (CDX-110) with GM-CSF Temozolomide, KLH	USA

(*Continued*)

Table 2. (Continued).

#	ClinicalTrials.gov Identifier	Title	Status	Phase	Diseases	# of Pts. Enrolled	Treatment	Locations
44	NCT02311920	Ipilimumab and/or Nivolumab in Combination with Temozolomide in Treating Patients with Newly Diagnosed Glioblastoma or Gliosarcoma	Active, not recruiting	I	Gliosarcoma Supratentorial Glioblastoma	32	Ipilimumab, Nivolumab, Temozolomide	USA
45	NCT03173950	Immune Checkpoint Inhibitor Nivolumab in People with Recurrent Select Rare CNS Cancers	Recruiting	II	Pediatric Brain Tumors	180	Nivolumab	USA
46	NCT04606316	Surgical Nivolumab and Ipilimumab for Recurrent GBM	Recruiting	I	Glioblastoma Grade IV Astrocytoma	60	Nivolumab, Ipilumab	USA
47	NCT02359565	Pembrolizumab in Treating Younger Patients with Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, Hypermutated Brain Tumors, Ependymoma or Medulloblastoma	Recruiting	I	Malignant Glioma	110	Pembrolizumab	USA
48	NCT02054806	Study of Pembrolizumab (MK-3475) in Participants with Advanced Solid Tumors (MK-3475-028/KEYNOTE-28)	Completed	I	Solid Tumor	477	Pembrolizumab	NA
49	NCT03389802	Phase I Study of APX005M in Pediatric CNS Tumors	Recruiting	I	Malignant Glioma of Brain	45	APX005M	USA
50	NCT04801147	Immunotherapy with Autologous Tumor Lysate-Loaded Dendritic Cells in Patients with Newly Diagnosed Glioblastoma Multiforme	Recruiting	I, II	Glioblastoma	76	Dendritic Cells Vaccine, Temozolomide	IT
51	NCT02017717	A Study of the Effectiveness and Safety of Nivolumab Compared to Bevacizumab and of Nivolumab with or without Ipilimumab in Glioblastoma Patients	Active, not recruiting	III	Recurrent Glioblastoma	530	Nivolumab, Bevacizumab, Ipilimumab	USA
52	NCT02658981	Anti-LAG-3 Alone & in Combination w/ Nivolumab Treating Patients w/ Recurrent GBM (Anti-CD137 Arm Closed 10/16/18)	Active, not recruiting	I	Glioblastoma Gliosarcoma Recurrent Brain Neoplasm	63	Anti-LAG-3 Monoclonal Antibody Nivolumab	USA
53	NCT02052648	Study of IDO Inhibitor and Temozolomide for Adult Patients with Primary Malignant Brain Tumors	Completed	I, II	Glioblastoma Gliosarcoma Malignant Brain Tumor	160	Indoximod, Temozolomide, Bevacizumab Stereotactic Radiation	USA
54	NCT02502708	Study of the IDO Pathway Inhibitor, Indoximod, and Temozolomide for Pediatric Patients with Progressive Primary Malignant Brain Tumors	Completed	I	Malignant Glioma of Brain	81	Indoximod, Temozolomide, Radiation Cyclophosphamide, Etoposide	USA
55	NCT01349036	A Phase 2 Study of PLX3397 in Patients with Recurrent Glioblastoma	Terminated	II	Recurrent Glioblastoma	38	PLX3397	USA
56	NCT03746080	Whole Brain Radiation Therapy with Standard Temozolomide Chemo-Radiotherapy and Plerixafor in Treating Patients with Glioblastoma	Recruiting	II	Malignant Glioma of Brain	20	Plerixafor, Temozolomide Radiation Therapy	USA
57	NCT03392545	Combination of Immunization and Radiotherapy for Malignant Gliomas (InSituVac1)	Unknown	I	Glioblastoma Glioma of Brainstem Glioma, Malignant	30	Combined immune adjuvants and radiation	CHN
58	NCT01204684	Dendritic Cell Vaccine for Patients with Brain Tumors	Active, not recruiting	II	Glioma Anaplastic Astrocytoma Glioblastoma	60	Autologous tumor lysate-pulsed DC vaccination, Tumor lysate-pulsed DC vaccination+0.2% resiquimod, Tumor-lysate pulsed DC vaccination +adjuvant polyICLC	USA
59	NCT01790503	A Phase 1b/2 Study of PLX3397 + Radiation Therapy + Temozolomide in Patients with Newly Diagnosed Glioblastoma	Completed	I, II	Newly Diagnosed Glioblastoma	65	PLX3397 Radiation Temozolomide	USA
60	NCT02829723	A Study of BLZ945 Single Agent or BLZ945 in Combination with PDR001 in Advanced Solid Tumors	Active, not recruiting	I, II	Advanced Solid Tumors	198	BLZ945 PDR001	USA

A: Australia; AU: Austria; CHN: China; DC: Dendritic Cell; ES: Spain; FR: France; GBM: Glioblastoma; IDO: indoleamine 2,3-dioxygenase; IR: Iran; IT: Italy; J: Japan; LAG3: lymphocyte activation gene-3; NA: not available; Pts: patients; RT: radiotherapy; SE: Sweden; TMZ: temozolomide; USA: United States; ZH: Zurich.

the existing clinical trials CPIs-based may depend on the intrinsic genome heterogenicity, immunosuppressive glioma microenvironment, and hitches in reaching the tumor site.

The tumor mutational load, namely glioma mutational burden (GMB), represents a critical prognostic factor in the CPIs therapy, also related to poorer survival [108–111]. These because immunotherapies are up to the expression of specific molecules, while the unpredictable mechanisms of DNA replication/repair result in ever-changing mutations and the potential loss of CPIs targets [111].

The further reason for failure consists in the intrinsic nature of the central nervous system as a site of immune privilege [112,113]. CPIs, as monotherapy, did not demonstrate efficacy due to the scarcity within the tumor microenvironment of active T cells with the concomitant secretion of immunosuppressive cytokines [114,115].

Furthermore, several shortcuts were designed aimed to overcome the BBB, including active efflux pumps, molecules directed against BBB biomarkers, and personalized administration routes [116]. Innovative mAb-delivering strategies, namely intra-arterial or intracranial injection, nanoparticle, and liposomal vectors are currently under investigation. The aim is to optimize drug delivery, intra-tumoral uptake, and boost immune pathways, generating host antitumor response and long-term lymphocytic memory [117–120].

2.1.2. Vaccines

Anticancer vaccination is included in the active immunotherapies and is designed to amplify the host immune response against glioma cells. The rationale for vaccines lies in the belief the central nervous system is an 'immunological sanctuary.' The brain immune privilege leads to the scarcity of T cells within the tumor site, in favor of immunosuppressive mechanisms [112,113,121]. Recent advances in translational medicine and immunogenomics allowed the detection of several tumor-associated antigens (TAAs) [122]. The presentation of TAAs, via anticancer vaccines, results in the transition of immunologically 'cold' glioma into an immunogenic one.

Peptide vaccines are composed of synthetic peptides directed to TAA leading to the stimulation of the anti-tumor immune cascade [123–125].

Rindopepimut (Rintega®, Celldex Therapeutics, Inc., Phillipsburg, NJ, USA) consists of a 14-mer epidermal growth factor receptor variant III peptide (EGFRvIII) combined with the keyhole limpet hemocyanin. Apart from the antigen-specific immune reaction, intended as the measure of the effectiveness of antitumoral vaccines, also cellular immunity has a pivotal role [126,127]. Vaccination with rindopepimut may activate CD4+ and CD8 + T cells against tumor cells exhibiting the EGFRvIII, found in 30% of GBMs [128–133] (Figure 3). It was employed in several phase II clinical studies, namely the ACTIVATE, ACT II–III, and ReACT. All of them reported an increase in overall survival and low levels of toxicity of the drug [132,134–137]. A further double-blind phase III trial, the ACT IV, proved on 745 newly diagnosed and surgically treated GBMs, the combination protocol including rindopepimut and TMZ [138]. Results were not satisfactory, and no survival benefits were reported. In fact, the median overall survival was of 20.1 and 20.0 months for the rindopepimut group and the control one, respectively. Despite the evidence of a vivid anticancer immune stimulation, the failure of the ACT IV protocol may be attributable to the downregulation of EGFRvIII-expressing glioma cells, resulting in the loss of rindopepimut efficacy.

The expansion of antigen-negative tumor cells, not recognizable by the activated T cells, leads to the malignant immune selection, glioma recurrence, and may be considered the main cause of failure of vaccine therapies [115,127,139].

Aberration of isocitrate dehydrogenase (IDH) occurs in about 80% of the glioma genetic landscape representing a histological landmark. The R123H IDH1 mutation is the most frequent, found in 70% of HGGs [140,141]. Peptide vaccines, comprising the R132H, were proved to induce the CD4+ activity by linking the major histocompatibility complexes (MHC) [142,143]. In 2021, Platten and colleagues demonstrated excellent immunogenicity and an increase in 2-years survival in 82% of HGGs patients treated with the R123H/IDH1 vaccine [144].

Recent epigenetics advances permitted the development of multi-peptide vaccines personalized against multiple TAAs [145]. The IMA950 was a specific HGGs peptide vaccine consisting of 11 different antigens. It was employed in many clinical trials. Despite a high level of safety and CD8 + T cell activation, no survival improvements were reported [146–148].

The personalized Neoantigen vaccine (NeoVax), built by personalized neo-TAAs plus multiple-epitope and engineered with adjuvant poly-ICLC (polyinosinic–polycytidylic acid and poly-L-lysine double-stranded RNA), was employed in a phase-I/Ib study for newly diagnosed GBMs. The NeoVax proved to boost the neoantigen-specific CD4+ and CD8 + T-cells recruitment within the glioma microenvironment, enhancing the local anti-tumor immune response [149]. Furthermore, a phase I clinical trial tested the NeoVax in a combined protocol with pembrolizumab, TMZ, and radiotherapy (#NCT02287428). The study is still recruiting.

SurVaxM, a peptide mimic vaccine, was designed to target the survivin, a cell-survival protein found in 95% of GBMs. This bond may boost the host antitumor immune reaction and stimulate glioma cell apoptosis. In a multi-center, phase II clinical trial the SurVaxM was injected in 63 patients affected by newly diagnosed GBMs, after surgery and therapy with TMZ (#NCT02455557). A 96.8% of vaccinated patients did not experience disease progression in the first 6 months and 93.5% of cases had an overall survival of more than 12-months. The immunogenicity of SurVaxM was confirmed by the identification of survivin-specific antibodies (IgG) and CD8 + T-cells recruitment [150].

A prospective, randomized, phase II clinical trial is investigating the SurVaxM with adjuvant TMZ for newly diagnosed GBMs ('SURVIVE') (#NCT05163080). This study is proposed to evaluate the clinical efficacy and survival benefits of the vaccine in 265 patients enrolled. The estimated completion date is 18 April 2024, but preliminary results reported promising immunogenicity of the SurVaxM with limited side effects.

In 2020 at Society for Neuro-Oncology Annual Meeting, the INOVIO Pharmaceuticals presented a phase I/II clinical trial designed to test the effectiveness and immunogenicity of INO-5401 and INO-9012 associated with cemiplimab (mAbs vs PD-1), radiation, and TMZ, for 52 GBMs (#NCT03491683). INO-5401

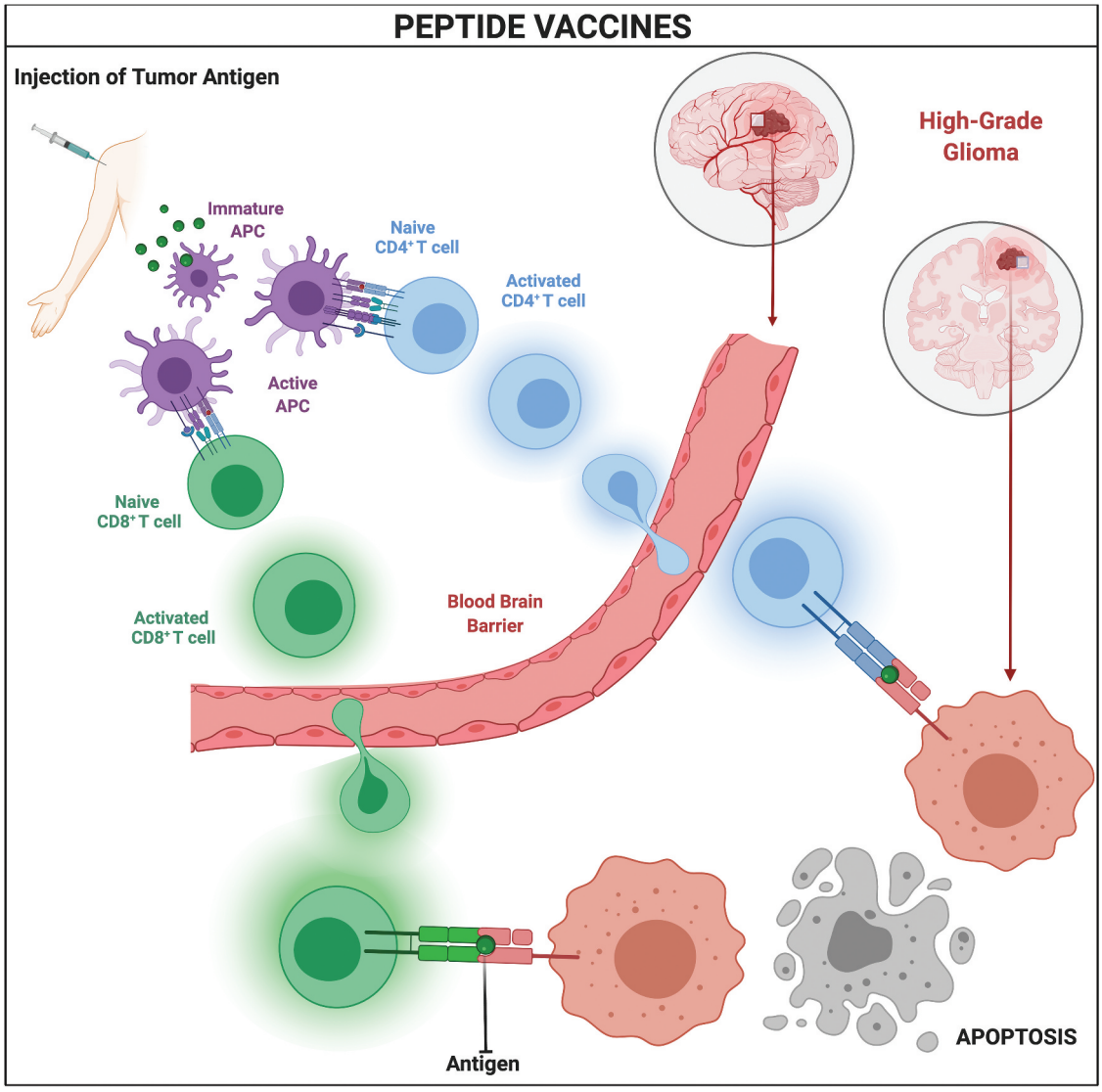

Figure 3. The figure reports the pharmacodynamics of peptide vaccines. Antitumoral vaccination consists of the injection of specific tumor-associated antigens which stimulate the host immune cascade. The activated CD4+ and CD8 + T cells cross the blood-brain barrier and selectively strike glioma cells inducing apoptosis.

consists of 3 DNA plasmids directed to Wilms tumor gene-1 (WT1), prostate-specific membrane (PSMA) antigens, and human telomerase reverse transcriptase (hTERT) gene. INO-9012 contains a DNA plasmid for transcription of interleukin-12 (IL-12). The scheduled completion date of the study is 30 June 2022.

A second-generation strategy was developed to reinforce the therapeutic efficacy, including the heat-shock proteins (HSPs) within the vaccine compound. HSPs act as intracellular chaperones in TAAs presentation and innate and adaptive immune activation [151–153].

HSP-peptide complexes (HSPPCs) were adopted in preclinical studies on mouse models and clinical trials [154,155]. In 2014, in a phase II clinical trial, Bloch et al. administered the HSPCC-96 vaccine to GBMs patients after surgical resection. The overall survival amounted to 42.6 weeks and no side effects were described [156].

Cell-based vaccines represent a promising strategy. They mostly consist of autologous dendritic cells (DCs) manipulated to present antigens and boosted the immune response [157,158]. The DCVax-Brain, composed of DCs and autologous glioma TAAs, was tested in phase I and II trials revealing few adverse effects [159,160].

The PerCellVac2, another multiple-epitope vaccine, was also employed in HGGs treatment. PerCellVac2 was made up of allogeneic blood cells combined with customized glioma antigens [161–163]. Its clinical efficacy is still debated.

NeoTAA-targeting vaccines demonstrated, in preclinical and clinical studies, encouraging results in converting the immunosuppressive microenvironment into an immune niche. The administration of vaccines in combined protocols with standard therapies showed enforced efficacy with low toxicity. The maximum immunogenicity of vaccines, assessable by the recruitment of immune mediators and T cells, was noticed for the rindopepimut. NeoVax, SurVaxM, and INOs are the most innovative compounds, now under investigation in future trials.

2.1.3. Oncolytic virotherapy

The oncolytic virotherapy employs self-replication-selective oncolytic viruses (OVs) competent to selectively infect cancer

cells, induce lysis, and stimulate a robust antitumoral immune cascade [27,164–166] (Figure 4(a,b)).

The double-stranded DNA oncolytic herpes simplex virus (oHSVs) was the first used in gene-based immunotherapy. oHSV was deactivated through the deletion of genes for the ribonucleotide reductase (ICP6) (Unique Long39) and the protein synthesis promoting factor (γ34.5) [167,168]. Since 2000, the oHSV1716, made by the inactivation of γ34.5s, was experimented with by intratumoral inoculation or as combined therapy with surgery and dexamethasone in GBMs treatment. Despite the low toxicity and evident viral replication within glioma cells, no efficacy in increased survival was reported [169–171].

oHSVG207, deleted for γ34.5 and ICP6, is the second-generation OVs. It was used as local administration in the surgical cavity or as adjuvant therapy with radiation therapy. Again, a good safety profile was demonstrated but survival resulted not improved [172–175].

The latest OV, the rQNestin34.5, was designed to express the ICP34.5 under the control of a nestin promoter. It showed better survival in animal models and no side effects in a clinical phase I trial (#NCT03152318) [176].

The DNA conditionally replicating adenovirus (CRAds) was created by the subtraction of genes that limit the linkage with glioma cells (E1A-B). The lack of E1A and E1B proteins inhibits the direct binding with cells expressing pRB and p53, respectively. DNX-2401, without E1A protein, selectively replicates in the Rb-deficient glioma cells, while the ONYX-015, deleted in E1B, targets the tumoral p53-deficient cells [177–181].

At the American Society of Clinical Oncology Annual Meeting in 2017, Lang et al. illustrated their results of a study on the DNX-2401 for recurrent HGGs, the aim of which was to prove the efficacy as monotherapy and the synergic effect in combination with the interferon (INF) γ [182]. In 2018, the DNX-2401 was tested as a local injection in phase I clinical trial recruiting 37 patients with recurrent GBMs. A 5-months increase in survival for the study group was reported (#NCT00805376) [183].

In 2020, Zadeh and his group conducted a phase II multicenter study (CAPTIVE-KEYNOTE-192) employing the intratumoral injection of DNX-240 (tasadenoturev) followed by intravenous pembrolizumab (200 mg every 3 weeks up to 2 years or until progression) for relapsing GBMs (#NCT02798406). Results

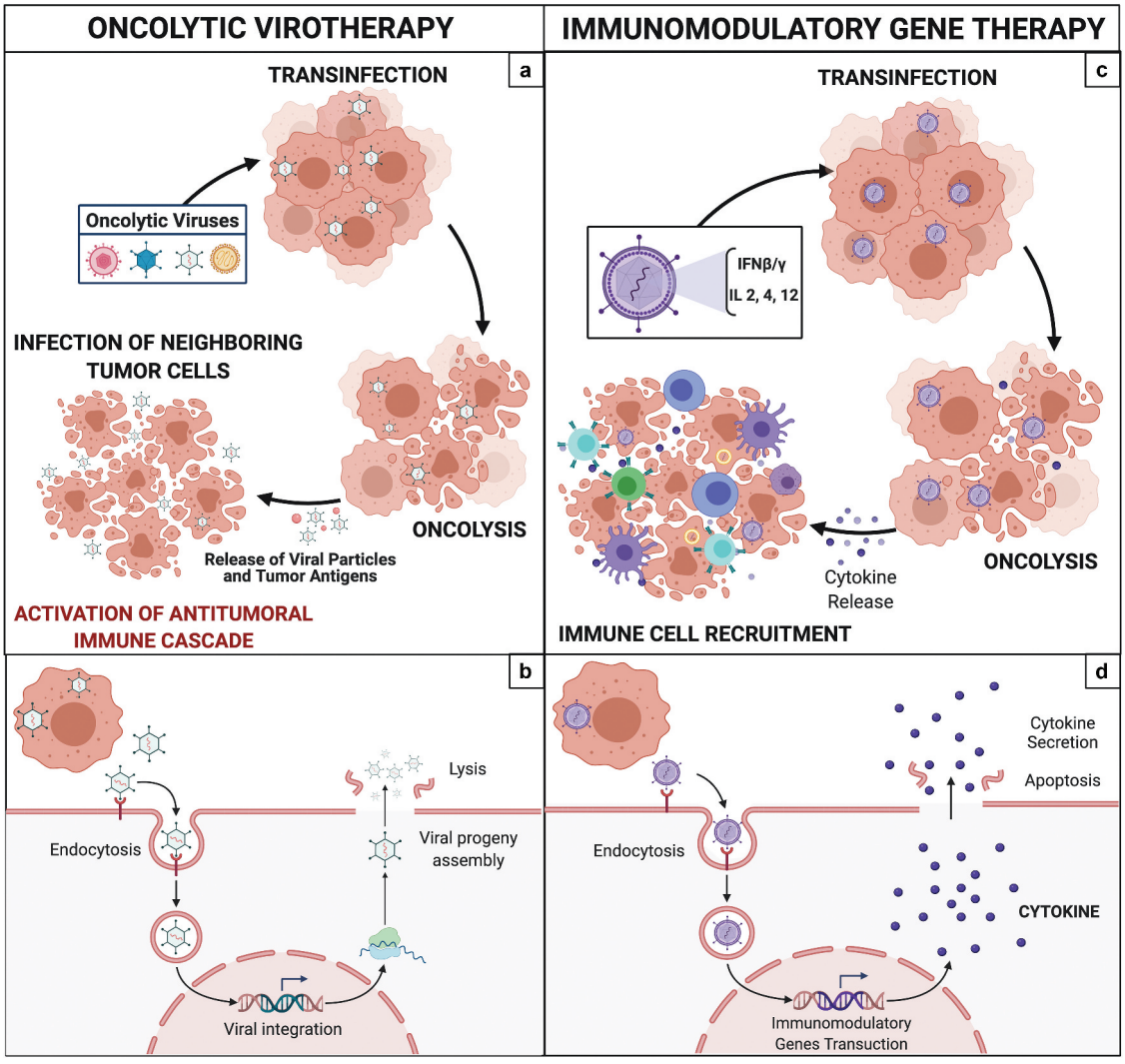

Figure 4. (a, b) The oncolytic virotherapy uses selective oncolytic viruses qualified to transinfect glioma cells through endocytosis. Viral integration into the cell genome leads to the viral progeny assembly and lysis of tumor cells. Oncolysis results in the release of viral particles and tumor antigens which activate the antitumoral immune cascade and infection of neighboring cells.

reported low toxicity, including headache and asthenia, with a median survival of 12.5 months [184]. A global, randomized phase III trial is scheduled.

The ONYX-015 was tried either via intratumoral injection within the surgical cavity [185,186], or combined with adjuvant chemotherapy (#NCT00006106), but no efficacy has been proven.

The enveloped RNA Measles Paramyxovirus (MV) was designed to express the mutated hemagglutinin envelope glycoprotein (Edmonston strain) able to bind the CD46 of HGGs cells [187,188]. MVs were furtherly manipulated to increase their oncolytic properties and selectivity for glioma cells. MVs were modified by expressing simultaneously the circulating carcinogenic embryonic antigen (CEA) [189,190], the IL-13 directed to IL-13Rα2 receptor on HGGs cells, or the Ab against the EGFRvIII [191–193].

The attenuated type 1 Sabin poliovirus was arranged through substitution of the internal ribosomal entry site (IRES) with the isoform from human rhinovirus type 2 to assemble the nonpathogenic poliorhinovirus (PVS-RIPO) [194–196].

PVS-RIPO was administered either alone or with lomustine or anti-PDL1 MAbs for pediatric or adult HGGs treatment. Results revealed high antitumor effectiveness, but several side effects were notified (#NCT03043391, NCT02986178, NCT03973879) [197–199].

The Newcastle Disease Virus (NDV) was manipulated to selectively target glioma cells, inducing lysis and apoptosis. Three oncolytic viral strains of the attenuated NDV were developed, namely the MTH-68, NDV-HUJ, and Ulster.

In 1999, Csatary and Bakács tested the MTH-68 in the treatment of a recurrent GBM, showing a reduction of glioma volume simultaneously with a clinical neurological improvement [200]. The same group, in 2004, employed the MTH-68 for the treatment of adult relapsing GBMs. They reported a high survival rate of 5–9 years in 4 patients, of all the 14 patients treated. No adverse event was described [201]. The rationale for the success of MTH-68 oncolytic viral treatment for HGGs may lie in the lytic viral capabilities in combination with immunomodulating properties. After administration, the MTH-68 selectively transinfects glioma cells, by attaching sialic acid receptors limited to cancer cells, and self-replicates [202,203]. It induces oncolysis, apoptosis, and enhances host antitumor immune response [202,204].

Freeman et al. tested the NDV-HUJ in 14 HGGs patients, 3 of which were long-survival [205]. In 2001, Ulster was administered to 11 patients affected by GBMs, after surgery and radiotherapy, and no substantial advantages were revealed compared to conventional therapy [206].

Reovirus, a double-stranded RNA virus, was utilized for the capacity to selectively infect glioma cells, overactive Ras signaling pathways, and induce apoptosis [207–209]. In 2014, Kicielinski and his group employed the Reolysin, as wild-type reovirus, in phase I clinical trial for GBMs treatment reporting an increase in overall survival up to 140 weeks [210]. In 2015, these findings led to the FDA approval of the Reolysin for HGGs therapy [211]. Additionally, the reovirus proved to detain a basic role in the activation of the immune cascade through the induction of DCs growth, the implementation of the proinflammatory cytokine, CD8 + T, and NK cell infiltration [212]. The reoviruses administration initiates the mechanism of tumor cell adaptation and immune escape, as the overexpression of PD-1/PD-L1 proteins to counter the immune activity. Additional studies are needed to test the association of reovirus virotherapy combined with anti-PD-1/PD-L1 MAbs [213,214].

Oncolytic virotherapy is a valuable second-line treatment for recurrent HGGs. The replication competent OVs are genetic payloads, capable of transinfecting glioma cells and inducing oncolysis. The apoptosis causes the release of TAAs, thus stimulating a robust immune reaction [27,164].

2.1.4. Immunomodulatory therapies

The immunomodulatory strategies, counted in the active approaches, include the immune-genes transferal and approaches targeted to the myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAM). All of these were projected with the purpose to strengthen the antitumor immune response and counteract mechanisms of glioma immune evasion.

The immunomodulatory genes are transferred via viral vectors and transcribed for immunostimulant ILs or IFNβ/γ [166,215–217] (Figure 4(c,d)).

IL-12 acts by recruiting lymphocytes and antitumoral factors within the tumor microenvironment [218–220]. The recombinant ADvs and γ34.5-deleted-HSV1 viruses, engineered for carrying the IL-12 gene, were administered to transinfect glioma cells in preclinical HGGs animal models [221–223]. The results were hopeful, demonstrating a decrease in cancer cell activity, but clinical trials are still underway.

Some clinical and preclinical studies used IL-4 as an immunostimulant factor for the recruitment of CD4+ and CD8+ cells [224–226]. Okada and Colombo both verified the usefulness of the IL-4-HSV-tyrosine kinase (TK) gene transmitted via viral vectors, followed by systemic ganciclovir, for refractory HGGs therapy. The efficacy of interleukins was also amplified by the activation of ganciclovir through the TK enzyme. High antitumoral activity and immune activation were revealed [226,227].

Furthermore, IFN-β/γ, delivered by viral carriers or nanoparticles, was used to enhance the host antitumor defenses via intratumor injection or systemically. Data from clinical studies agreed with the premises and demonstrate a strong activation of the immune cascade [228–232].

Natsume and colleagues tested the efficacy of INF-β in rats or murine HGGs models. Their results showed a reduction in tumor growth, with strong antitumoral activity within the tumor microenvironment [233]. Yoshida and his group employed the INF-β in human GBMs treatment and reported a good safety profile, low level of toxicity, and histological evidence of immune cells recruiting [234,235].

IFN-γ was administered as a combination protocol with the tumor necrosis factor-α to enhance immunization against tumor and cytokine recruitment. Results are still being evaluated [236–239].

A further arm of immunomodulant therapies consists of the approaches focused on the MDSCs [240]. MDSCs are immature myeloid cells which exert a strong immunosuppressive activity within the tumor microenvironment, regulating lymphocyte recruitment and mechanisms of immune evasion [241–244].

MDSCs secrete immunosuppressive cytokines, such as the IL-10 and TGF-β, stimulating the reactive oxygen species and nitric oxide and causing the death of immune cells [245,246]. Evidence of the MDSC-mediated immunosuppression provided the basis for the design of tailored strategies aimed to target the glioma MDSCs. The main techniques developed include the inhibition of myeloid cell polarization to MDSC; the recruitment and depletion of MDSCs; and the fostering of their immunosuppressive activity [247,248].

Huang et al. wide described the involvement of VEGFR2 not only in the tumor angiogenesis but also in the myeloid differentiation to MDSCs [249]. The altiratinib, a VEGFR2 inhibitor, combined with BVZ was tested in preclinical trials on mouse models demonstrating immune-suppressive functions [250]. IL-8 acts as a chemotactic agent for the MDSCs in the tumor microenvironment operating on the C-X-C motif chemokine receptors [251]. Huang and his group also proved that ABX-IL8, a mAbs vs IL-8, inhibits vasculogenesis and MDSC recruitment [252].

The galectin-1 (Gal-1) links the β-galactosides and regulates T cell activity and VEGF-A functions [253,254]. In 2016, Van Woensel and colleagues tested the intranasal administration of nanoparticles engineered with siRNA directed against Gal-1 in GL261 glioma mouse samples. They reported a considerable reduction of MDSCs in the tumor microenvironment [255].

TAMs regulate the immune response and take up a fundamental function in glioma development [256,257]. Therapeutic approaches directed against TAMs were designed with a double purpose: to inhibit TAMs recruitment and reverse the M1/M2 ratio.

TAMs recruitment can be avoided by hindering the secretion of inflammatory chemokines from glioma cells. The CCL2-CCR2 and CXCL12-CXCR4 axes stimulate the infiltration of monocytes and macrophages, establishing an immunosuppressive tumor microenvironment [258].

Anti-CCL2 inhibitors were tested in phase I/II clinical trials. Although demonstrating a good safety profile, clinical implementation is still far [259–261]. A CXCR4 antagonist was analyzed in combination with Bevacizumab for HGGs patients showing no therapeutic effects [134], but concurrently with TMZ the survival increased by 10 months [261]. Plerixafor, a CXCR4 antagonist is under evaluation in a recruiting phase II clinical trial, combined with the standard protocol (#NCT03746080).

Strategies of reprogramming polarization to the pro-inflammatory M1phenotype employ the anti-CD47 molecules. CD47 binds the signal-regulatory protein-α (SIRPα) leading to the inhibition of tumor cell phagocytosis macrophages-mediated. Blockage of CD47 results in an increase in antitumor M1 activity and improves prognosis in animal glioma models [262–264].

Toll-like receptors (TLRs) are surface molecules expressed on macrophages [265]. TLR agonists, competent to activate the M1 shifting, are being tested as adjuvants in glioma patients (#NCT03392545, #NCT01204684).

On the other hand, further strategies focused on reducing pro-tumorigenic M2 TAMs. The macrophage colony-stimulating factor (M-CSF) is potentially able to shift the macrophages to the M2 subtype, influencing tumor proliferation and immune resistance [266,267]. On this rationale, the BLZ945 and PLX3397, CSF blockades, were administered to glioma patients. Results reported inhibition of TAMs-M2 phenotype but disproved the therapeutic effect also in combination with standard of care or anti-PD-1 antibody (#NCT01349036, #NCT01790503, #NCT02829723) [268,269].

Surface receptors of M2, such as CD163 and CD204, may be potential therapeutic targets and markers of malignancy and poor prognosis but are not yet explored in clinical trials for HGGs [270–272].

Although the preliminary and preclinical contrasting outcomes, the experimented immunomodulatory strategies demonstrated encouraging and favorable results. The genome reprogramming in the glioma cells strives to yield apoptosis through the transcription of antitumoral mediators. Engineered genes are delivered to target cells via vehicles, viral or not, which directly attach glioma cells. Future studies are focusing on the search for new transport systems aimed at optimizing the bioavailability, the biodistribution through BBB, and limiting the toxicity.

2.2. Adoptive immunotherapies

2.2.1. Engineered T cells

The T cell-based approaches employed autologous T-cells, levied with leukapheresis, engineered by viral transduction of specific tumoral genes, ex vivo expanded, and then systemically administered. The transinfected genes were the chimeric antigen receptor (CAR) and the transgenic T cell receptor (TCR) genes [273–275]. CARs, expressed on T cells, are designed to directly bind ligands present on the surface of the tumor, whereas TCRs directly target the MHC. Both boost the activation of antitumor immunity [276–282] (Figure 5).

CARs selected in the HGGs treatment were as follows: EGFRvIII, IL-13 receptor a2 (IL-13Ra2), human epidermal growth factor receptor 2 (HER2), and erythropoietin-producing hepatocellular carcinoma A2 (EphA2) [282–291].

Despite preclinical studies on CART-EGFRvIII cells demonstrated to limit the tumor growth [292,293], clinical trials have not yielded satisfactory results. The not negligible side effects were a further weakness [294].

The IL-13Ra2 link to the IL-13 triggers the proinflammatory intracellular signaling through the JAK/STAT pathway [295]. In a clinical study conducted by Brown et al., the IL13Rα2-targeted CAR T cells were locally injected through a caterer after the removal of a supratentorial GBM. There was no local relapse, but the tumor metastasized into the leptomeningeal spinal space. Recurrence was treated again with IL13Rα2-targeted CAR T cells via an intraventricular catheter. The patient survived for a further eight months [282].

HER2 was expressed on 80% of HGGs and involved in glioma progression mechanisms [296]. HER2-specific CAR T cells showed high anticancer efficacy in GBM immunodeficient mice [289]. In 2010, Ahmed and colleagues conducted a phase I trial on HER2-CAR T cells for human HGGs. The HER2-CAR T cells were identified in the peripheral blood for one year after infusion, but no significant survival improvements were described [297]. Despite

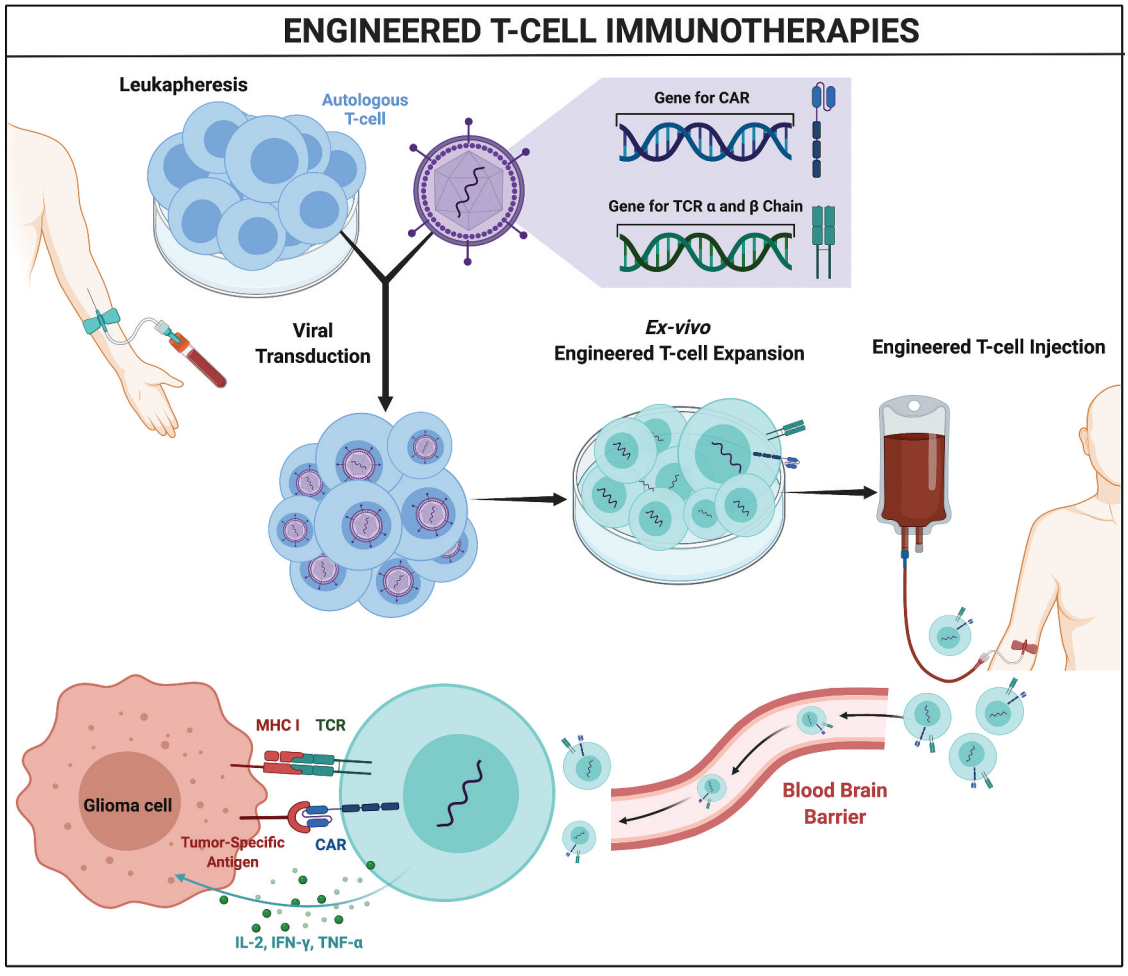

Figure 5. The figure illustrates the engineered T-cells' mechanisms of action. Via leukapheresis, the autologous T-cells are levied and in vitro engineered with viral vectors which include the chimeric antigen receptor (CAR) genes, prepared to bind tumor-specific antigens, and the transgenic T cell receptor (TCR) genes directed to the Major Histocompatibility Complex (MHC). Engineered T-cells are ex-vivo expanded and injected. They cross the blood-brain barrier and as a result of cell-binding boost the antitumor immunity.

TNF: Tumor Necrosis Factor.

the reported good safety profile, the greatest risk lies in the possibility of targeting healthy tissues expressing the HER2 [298].

In 2022, Majzner and his group published the preliminary results of phase I clinical trial on the disialoganglioside (GD2)-CAR T therapy for children and young adults with H3K27M-mutant diffuse intrinsic pontine or spinal glioma (#NCT04196413). The GD2-CAR T cells, administered via retroviral vectors, demonstrated promising efficacy and low side effects. Inflammatory mediators were found increased in the plasma and cerebrospinal fluid. Patients experienced also neurological and radiological advancements [299].

TCR genes programming involved the isolation of the α and β chains and the development of specific receptors which selectively interact with tumor MHC on glioma cells resulting in T cell activation and oncolytic effect [300–303].

The tremendous advances in somatic cell biotechnologies are reflected in the realization of T cell engineering. As for other oncological fields, the CAR T-based clinical trials, especially those directed toward the EGFRvIII, yielded promising results also for HGGs treatment. The overall level of evidence of the effectiveness of efficacy of the CAR and TCR engineered T cells therapies is extremely encouraging but still inadequate to be assessed as usable in daily clinical practice [279,283–289,291,294,297,304–307].

2.2.2. NK cells and mixed strategies

NK cells were used in the adoptive immunotherapies in different ways [308].

Allogenic NK cells may be transplanted to avoid MHC-mediated surveillance expressed on glioma cells and control the immune antitumoral effect [309–312]. Heterologous NK cells can also be transferred by carrier exosomes to facilitate drug penetration.

In 2017, allogenic cord blood NK cells were retrovirally transduced to incorporate genes for the TGF-β-dominant-negative receptor II (DNRII). DNRII makes NK cells exempt from the anti-immune-mediated effect of TGF-β within the tumor microenvironment [313].

Furthermore, the engineering of NK cells by means of CAR genes, especially directed against EGFRvIII, was studied [314–316].

The antibody-dependent cell-mediated cytotoxicity (ADCC) approach utilizes specific Abs able to bind glioma antigens and activate NK cells. The Ab fragment crystallizable (Fc) ligates the NK receptors and the Fab portion binds antigens

on the glioma cell surface. The explored glioma antigens were as follows: EGFR, CD16 (FcγIIIA), and NK Group 2D (NKG2D) [317].

Another indirect strategy provides for the use of Abs directed against the NK Immunoglobulin-Like Receptor (KIR) which naturally inhibits the activity of NK cells. The antibody-mediated KIR censure increases the NK cell function and glioma death [308].

NKT cells express the properties of both NK and T cells. Particularly, CD1d-restricted NKT cells regulate the immune escape mechanisms within the glioma microenvironment [318,319]. The expansion and transplant of these cells, cultured with dendritic cells and the α-galactosylceramide, was proved to be a potential strategy [320,321].

Autologous Lymphoid Effector Cells Specific Against Tumor cells (ALECSAT) (Cytovac A/S, Hørsholm, Denmark) is a 26-days multimodal therapy based on the manipulation of autologous monocytes, CD4+, CD8+, and NK cells. These were isolated, loaded with 5-aza-2′-deoxycytidine which induces expression of cancer antigens, activated, and then injected. Activated lymphocytes were directed against the tumor cells, whereas cancer cells antigen-missing were targeted by NK cells [322].

The NK cell-based and hybrid strategies are intended to replace the immune cells which are pent up within the tumor microenvironment.

Despite favorable premises and a good safety profile, no study yet confirmed the clinical efficacy of the NK and mixed adoptive therapies. The main translational challenges still lie in the necessity to overcome the immune escape mechanisms, such as the lack of TAAs or the overexpression of MHC, and the immunological resilience of HGGs [309–312].

3. Conclusion

The novel immune-based therapies aim to overcome the resilience of malignant brain tumors to conventional treatments.

Besides TMZ, active immunotherapies include a key MAb, namely the BVZ, an anti-VEGF monoclonal IgG1approved as a second-line treatment.

Peptide and cell-based vaccines failed to show a significant increase in survival rate while demonstrating a substantial activation of the antitumor immune response.

Amid the gene-based technologies, oncolytic virotherapy proved to be the most reliable and safe approach. oHSVs, CRAds, PVS-RIPO, MV, NDV, and reoviruses were designed to selectively lyse the glioma cells, simultaneously boosting the immune system against the released TAAs. The delivery of immunomodulatory genes via viral vectors was designed as an integrative treatment to modulate and amplify the immune response within the tumor microenvironment.

Adoptive immunotherapies based on T, NK, and NKT cells had promising results. The detection of specific TAAs, suitable as potential targets, led to improving the design of the tailored CAR and TCR T cells, which have been accordingly made more effective.

Despite the increasing pieces of evidence of their feasibility and safety, immunotherapies were not yet validated as alternative strategies for recurrent HGGs.

Further clinical trials are needed to definitively move the personalized immune approach from the bench to the bedside.

4. Expert opinion

4.1. Mutational burden and immune landscape in glioma microenvironment

In the last decade, numerous studies have focused on the development of innovative strategies for HGG treatment, intending to elude mechanisms of immune evasion.

The brain immune privilege, the heterogeneity of the glioma genetic landscape, the persistence of cancer stem cells, and the immunosuppressive microenvironment account for the resistance of HGGs to standard treatments [12,15,20].

Despite the central nervous system being described as an ‘immunologically cold’ sanctuary, the identification of meningeal lymphatic drainage, namely the ‘glymphatic system,’ provided the theoretical basis for immunotherapy [323,324]. The glymphatic system regulates the brain immune surveillance and the lymphocytes flow but, concurrently to glioma growth, gets locked [325,326].

The restoration of intracranial immune function, via active or adoptive immune approaches, may result in an advantageous strategy to ‘warm-up’ the tumor niche and turn the cold glioma into a hot immunogenic one.

The heterogeneity of the glioma genome, i.e. the GMB, is considered a potential biomarker for prognosis and response to immunotherapies [110,111]. GMB is described as the set of mutations and somatic protein-coding base substitutions. Several studies have demonstrated a direct correlation between high GMB, glioma malignancy, and worse survival [327,328].

Furthermore, the GMB was proven to influence the inflammatory response and immune recruitment within the tumor microenvironment, affecting the efficacy of immunotherapies [111,329,330].

The immunosuppressive microenvironment inhibits the host antitumoral response and sustains the adaptation and proliferation of glioma cells. The detection of the immune niche composition is pivotal to predicting the mechanisms of tumor resistance.

Regarding the lymphocytes compartment, glioma cells evade the active immune cells’ recruitment, and the CD4 + and CD8 + T, and NK infiltrating cells were found to be scarce. The seizure of T cells, within the bone marrow, is forced by the loss of the sphingosine-1-phosphate receptor, an essential receptor for the lymphocyte chemotaxis [331]. On the contrary, the Tregs were the most expressed. Tregs contribute to the immune modulation and reduction of the T and NK cells, leading to the failure of the immune antitumoral response [332].

TAM, recruited in the perivascular microglia, sustain the glioma proliferation and invasion. The high expression of CD163 and CD204 revealed a strong polarization toward the M2 immunosuppressive phenotype within the tumor microenvironment. M2 macrophages stimulate tumor expansion, through the release of growth factors, VEGF, IL6, and IL10, and induce T cell apoptosis [114,115,266,333–335].

Mesenchymal stem cells were found in animal and human GBMs, enhancing the mechanisms of invasion and progression of glioma. The existence of glioma stem cells in the periphery promotes self-renewal and aberrant cell growth.

MDSCs are myeloid cells expressed at the tumor site. MDSCs are competent to block T-cell activity, simultaneously boost the Treg recruitment, and play a fundamental role in tumor angiogenesis through the presentation of VEGFR2. MDSCs are also to be intended as biomarkers for tumor staging and therapeutic response to chemo- and immunotherapy [240,243,336].

Accordingly, the lymphodepletion, the prevalence of Tregs, M2, and MDSCs negatively affect the prognosis and constitute premises for the failure of standard treatments and T-cell-based immunotherapies. On this rationale, the acknowledgment of immune infiltrates within the microenvironment may be the starting point for planning target treatment, aimed at increasing or switching off specific immune pathways, ultimately destroying the immunosuppressive barriers.

4.2. Constraints and future perspectives

Besides the intrinsic malignant nature of GBM, some specific pharmacodynamic limitations prevent the success of immunotherapies.

The first point is the choice of the most efficient route of delivery of the drug, which must be able to cross the BBB. Novel biocompatible small carriers, such as nanoparticles and liposomes, were designed for this purpose [337].

An alternative option is the prophylactic weakening of BBB via mannitol, osmotic compounds, or, more recently, ultrasounds. Low frequency focused ultrasounds were delivered trans-cranially in precise sites of the brain to reversibly destroy the BBB, thus allowing a successful and satisfactory penetration of the drug [338,339].

A cutting-edge technique proposed by Patel and colleagues in 2020 employs the periosteal and temporoparietal fascial flaps (TPFFs) to skip the BBB. TPFFs can be easily rotated and, because their vascularization comes from the external carotid artery, they completely bypass the hindrance of the BBB. TPFF transposition to the surgical wall after tumor removal allows the drug distribution to the remaining tumor cells [340].

Moreover, forthcoming challenges are directed toward innovative strategies striving at scanning the efficacy of immunotherapies, earlier during treatment. Clinical trials should be integrated with valid immune tracking, aimed to detect the precocious effects of immune agents and response to therapy.

Immune monitoring is feasible via neuroimaging tools, such as radiomics or positron emission tomography to assess the inflammatory metabolism, or blood and tissue analyses [341,342].

Measuring the host immune response through blood tests is advantageous. Peripheral T cell activation and clonal expansion, secretion of proinflammatory chemokines, ILs, and IFN reflect the patient's response to treatment [139,341,343]. This evidence teases out information about the prognosis and whether needs to change or implement the ongoing treatment.

Monitoring of therapeutic efficacy may be noticed through the detection of intra-tumoral markers. Amid these, the glioma TCR repertoire was evaluated in many clinical studies which reported contrasting survival results [344,345].

The development of novel administration routes, tools for monitoring the therapeutic response, and the concurrent evolution in the design of more effective and safe vehicles represents the pivot for the full integration between the first-line protocols and the personalized immunotherapies.

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